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Intensification of Wild Silkworm *Attacus atlas* Rearing (Lepidoptera: Saturniidae)

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ABSTRACT

Study on intensification program through reproduction and feeding management was conducted for six months to evaluate the growth and reproductive performance of wild silkworm, *Attacus atlas*. The experiment was divided into two phases: 1) the first phase was conducted on mating management, by applying different times of mating duration of 3, 6, 12, and 24 h as treatments to assess eggs production. In this phase, a completely randomized design was used, with 4 treatments and 5 replications; 2) the second phase was on feeding management, a completely randomized design method (2 x 2) was used, including feed (guava and walnut leaves) as first factor, and the second factor was feeding times (3 and 4 times/day) with 5 replications. The results obtained from the first experiment showed that different duration of mating significantly (P<0.05) affected the number of eggs produced. Based on time efficiency 6 hours mating duration produced higher egg production (226 egg/cycle), as well as hatchability (95%) than those of other mating duration. In the second experiment, it was found that there was interaction between feed types and feeding frequencies which significantly (P<0.05) affected feed consumption, feed digestibility, body weight gain, diameter of larvae and mortality rate. In conclusion, the optimum and efficient duration sugested for mating is six hours resulting high egg production. The walnut leaves feed has better effect on the first and second instar of larvae, while the guava leaves affect the following instars. In terms of production, four times of feeding frequency per day has higher productivity and is recommended in the cultivation management of *A. atlas*.

Key words: Attacus atlas, feeding management, intensification, mating management

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi penerapan program intensifikasi pemeliharaan *Attacus atlas*. Penelitian dibagi dalam dua tahap: 1) tahap pertama adalah manajemen perkawinan yang dilakukan berdasarkan lama perkawinan yang berbeda yaitu 3, 6, 12, dan 24 jam dan dinilai pengaruhnya terhadap produktivitas dan performa telur. Penelitian ini menggunakan rancangan acak lengkap dengan lama perkawinan sebagai perlakuan dan lima ulangan; 2) tahap kedua adalah manajemen pakan yang menggunakan rancangan acak lengkap pola faktorial 2 x 2, yang terdiri atas faktor pertama, yaitu jenis pakan (daun jambu dan kenari); faktor kedua, adalah waktu pemberian pakan per hari (3 dan 4 kali). Hasil penelitian fase pertama menunjukkan bahwa perbedaan lama kawin mempengaruhi (P<0.05) jumlah telur dan daya tetas telur. Hasil penelitian fase kedua mengindikasikan adanya interaksi antara jenis pakan dan frequensi pemberiannya terhadap parameter produksi *A. atlas*. Terdapat perbedaan nyata (P<0.05) konsumsi pakan segar, kecernaan pakan, pakan tercerna, pertambahan bobot badan, pertambahan diameter badan larva, dan mortalitas pada waktu dan frekuensi pemberian pakan yang berbeda. Disimpulkan bahwa lama perkawinan 6 jam menghasilkan jumlah dan daya tetas telur yang lebih tinggi (226 butir/induk/siklus dan 95%) dibandingkan perlakuan lainnya. Daun kenari memberikan hasil yang lebih baik pada instar I dan II, sementara daun jambu memberi hasil yang baik pada instar ke III dan sampai ke V. Frekuensi pemberian pakan 4 kali per hari meningkatkan produktivitas ulat sutera *A. atlas*.

Kata kunci: Attacus atlas, intensifikasi, manajemen pakan, manajemen perkawinan

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INTRODUCTION

Attacus atlas was a wild silkworm species which was found in almost in all parts of Indonesia (Wolfgang *et al*., 2010), still considered as wild animals, and very much depending on the available feeds around their habitat. This insect was categorised as polivoltin and polifagus, potentially could be cultivated because of their capability to consume a large varieties and types of feeds. Several feeds which were common to *A. atlas*, including tea leaves, soursop, avocado, cinnamon, bitterness, ylang-ylang, dadap, cloves, sengugu, turmeric, mango, kaliki, jatropha (Indrawan, 2007; Adria, 2010). *A. atlas* can produce cocoons (Faatih, 2005), that could be processed for good quality silk, however, the insect has not been intensively cultivated yet. Due to environmental changes and effects, mortality rate of *A. atlas* was very high, which may reach 90% (Situmorang, 1996). The main natural problems related to environmental factors that can not be controlled such as temperature, humidity, rainfall, light, types of feed available and diseases caused by parasites and predators were suggested as factors associated with such high mortality (Indrawan, 2007). Therefore, one of the alternative solutions to improve productivity is to apply an intensive management by keeping the insect in appropriate housing system, feeds and suitable environmental conditions.

In terms of reproductive performance, cultivation of *A. atlas* often encountered some natural problems including the unbalanced number of males and females imago in the population for fair matings, the shorter life cycle of males than females, and some of eggs produced are infertile. Therefore, optimum use of imago in mating systems, appropriate feed and feeding through intensive management may improve reproductive performance of *A. atlas*. Information on feeding and reproduction management of *A. atlas* were still rare, therefore, study on this aspect was necessarily of importance.

MATERIALS AND METHODS

The study was devided into 2 phases, including mating management and feeding management. The experiments were conducted for six months from September 2012 to February 2013, located in the Laboratory of Metabolism, Faculty of Veterinary Medicine, Bogor Agricultural University. Materials used were wild silkworm *A. atlas*, guava, and walnut leaves as feed sources.

A completely randomized design was used in the first experiment, by applying 4 different mating times (3, 6, 12, and 24 h) as treatments with five replications. Twenty moths (10 males and 10 females) were used. In the second experiment, a completely randomized design with 2 factors (types of feeds and feeding frequency) with 5 replications was applied. Guava and walnut leaves were given to animals in the frequency of feeding of 3 and 4 times per day. The total numbers of larvae used were 300 larva's, of which put in 20 experimental units with 15 larva's per unit.

Procedures and Data Analysis

This study was commenced with the collection of cocoons from tea plantation in Purwakarta. Cocoons were placed in a gauze cage 50 x 50 x 50 cm^2, and the moths were caged in pairs. The observed variables during the first phase included: 1) laying period, is the time required by female moth to oviposite her eggs; 2) eggs production counted as the total eggs produced by a female moth during her production cycles; 3) incubation time or time needed for incubation (in days), egg hatchability is the percentage of eggs hatched relative to the total eggs produced; and egg weight.

The variables measured in the second phase of experiment were done in each cycle of instar, included:

1) Feed intake= Σdaily feed intake
   Daily feed intake= {weight of given fresh leaves – [weight of residual leaves x correction factor]}/ number of larvas;
2) Feed digestibility= ([dry matter consumption – faeces dry matter]/ dry matter consumption) x 100%;
3) Digested feed= feed digestibility x fresh feed consumption;
4) Body weight gain= body weight at the start of instar – body weight at the end of instar;
5) Average diameter gain at anterior, middle, and posterior part of body, and mortality= ([number of larva at the start of instar – number of larva at the end of instar]/ number of larva at the start of instar) x 100%.

Data obtained from this study were analyzed using analysis of varianced (ANOVA). Duncan least significant mean test at α= 0.05 was used to assess the difference between treatments in order to determine the best mating and feeding management for application.

RESULTS AND DISCUSSION

Environmental Conditions

Temperature, humidity, and light intensity were measured to present the environmental condition during the study. The results showed that the average room temperature was 27.44±0.38 ^oC in the morning, 28.21±0.45 ^oC in the afternoon, and 27.52±0.52 ^oC in the evening, and the minimum and maximum temperature was 26.2 ^oC and 28.9 ^oC, respectively. Humidity in the morning, afternoon, and evening was 83.93±3.37%, 75.33±6.57%, and 82.42±5.14% respectively, with a minimum humidity of 56.9% during the day time and 91.7% in the evening. The morning light intensity was 0.009±0.004 Klux, 0.015±0.008 Klux during day time, and 0.008±0.017 Klux in the evening. Minimum light intensity occured in the evening (0.001 Klux) and the maximum occured in the afternoon (0.029 Klux). These overall conditions were still favorable for *A. atlas* to express good performance.

Mating Management

Female moths of *A. atlas* could produce eggs within the mating duration of 3, 6, 12, and 24 h. The results presented in Table 1, showed that the length of mating times did not significantly affect the average of laying period of 5.00±0.65 d. The laying period with mating time longer than: 3 h was 4.60±0.55 d, 6 h was 5.20±0.84 d, 12 h was 5.20±0.45, and 24 h was 5.00±0.63 d, which was relatively similar amongst treatments. According to Singh *et al*. (2003), the similar results indicates a good prospect required for cultivation process. The moth of *A. atlas* used chemical compounds such as pheromones to do their sexual communication with each others (Goswami & Singh, 2012). The antenna of males have an important function to detect the pheromones released by females as a signal and chemical cues to attract the males for copulation. The sexual activities was performed by nature, and then, the female moths will produce eggs (Jurgen *et al.,* 2005; Triplehorn & Johnson, 2005; Grater *et al*., 2006; Syed *et al*., 2006).

The trend of laying patterns in Figure 1 showed that egg laying at different mating duration indicated a declining pattern along with the longer mating duration during the days of mating activities. These figures may be associated with the decreasing of moth energy for mating within the period of 4-6 d (Goswami & Singh, 2012). After matings and egg producing, the females would do the oviposition, and then the last activity was laying eggs. The number of eggs produced was significantly affected (P<0.05) by mating duration. The results showed that the increase of egg number produced was in line with the increase of mating duration (3, 6, 12, and 24 h). Female moths mated for 24 h produced the highest number of eggs (288.40±77.63 eggs/cycle), followed by 245.00±50.70 eggs of 12 h; 225.80±24.30 eggs of 6 h; and the lowest was 155.60±48.75 eggs of 3 h mating duration. Egg production between 6, 12, and 24 h mating time was similar but the difference was found between the 3 treatments with those of 3 h mating time. The number of eggs produced were between the range of 155-288 eggs/cycle. In average, this result was not significantly different from the previous study (Nazar, 1990), of which female moths produced 286 eggs/cycle.

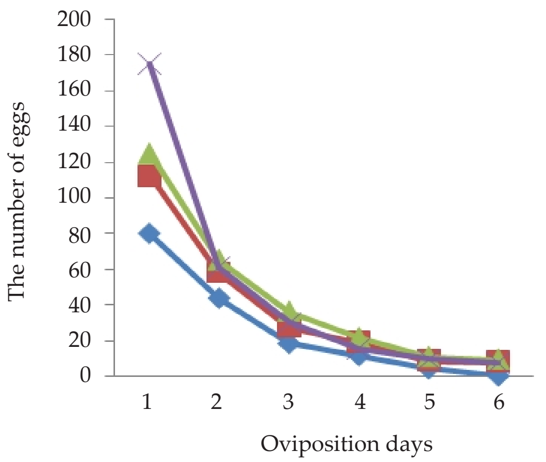

Figure 1. The reproduction pattern of *A. atlas* at different length of mating times. -♦- : 3 times, -■- : 6 times, -▲- : 12 times, -x- : 24 times.

The results from this study indicated that incubation time was not affected by the length of mating time; for each mating duration of 3, 6, 12, and 24 h, time spent by females for hatching eggs was 9.91±0.93, 9.57±0.99, 9.94±1.14, and 9.77±0.59 d respectively, with the average of 9.80±0.87 days/cycle. The figures were varied between treatments and was relatively different from the results of Adria & Idris (1997) of 7-13 days/cycle. Egg hatching period was naturally influenced by temperatures during the incubation process, the ecdysis and juvenile hormones (Triplehorn & Johnson, 2005; Woods, 2010) and also affected by the differences of individual embryo formation process which needed 7-8 d.

The length of mating duration significantly (P<0.001) affected hatchability. There was no differences in hatchability between mating times of 6, 12, and 24 h, however, a significance different was found between the three treatments with that of 3 h. The highest percentage of hatchability(96.41±2.08%) was found on insects mated for 24 h as compared to the insects mated for 3 h (82.06±5.18%). Adria & Idris (1997) reported that the egg hatchability of *A. atlas* in nature was 72.06%. Those differences may be due to environmental conditions during hatching process including egg storage, ecdysis and juvenile hormone (Triplehorn & Johnson, 2005). Long period of matings may influence the numbers of sperms stored by females which would increase eggs fertility. The similar result of egg weight among treatments may be associated with feed and feeding management, since the weight of eggs is basically more influenced by the quality of feed consumed by female moths during larvae phases.

Table 1. Laying period (LP), eggs number (NE), egg incubation time (IT), egg hatchability (EH), and egg weight (EW) of *A. atlas* with different mating duration

Variables	Mating period (h)				Average
	3	6	12	24	
LP (days)	4.60 ± 0.55	5.20 ± 0.84	5.20 ± 0.45	5.00 ± 0.63	5.00 ± 0.65
NE (eggs)	155.60 ± 48.75^{b}	225.80 ± 24.30^{ab}	245.00 ± 50.70^{a}	288.40 ± 77.63^{a}	228.70 ± 71.89
IT (days)	9.91 ± 0.93	9.57 ± 0.99	9.94 ± 1.14	9.77 ± 0.59	9.80 ± 0.87
EH (%)	82.06 ± 5.18^{B}	94.89 ± 1.23^{A}	95.28 ± 0.82^{A}	96.41 ± 2.08^{A}	92.16 ± 6.57
EW (mg)	6.90 ± 0.20	7.00 ± 0.70	6.80 ± 0.20	6.90 ± 0.60	6.90 ± 0.43

Note: Mean in the same rows with different small superscript differs significantly (P<0.05); mean in the same rows with different capital superscript differs significantly (P<0.01).

Feeding Management

The results indicated that types of feeds and feeding frequencies significantly (P<0.05) affected the feed consumption of *A. atlas* larvae (Table 2). The large amount of daily feed consumption was found at the first instar (I1) (0.317±0.015 g/d) and the second instar (I2) (0.459±0.015 g/d) on larvae given fresh walnut leaves for 4 times daily. Whereas, at instar 3 (I3), and instar 4 (I4), feed consumption was gradually increased (1.000±0.010 g/d) (1.291±0.132 g/d), on larvae given guava leaves for 4 times. Larvae of *A. atlas* preferred fresh feeds. According to Hamamura (2001), good quality of moistures, protein, fat and fiber content leaves would increase feeding activity and feed consumption of *A. atlas*. Guava leaves have rough structure, and is preferred by insects than walnut leaves. Young larvae (instar 1-4) of *A. atlas* preferred walnut leaves with soft structure. This results is supported by Vonny & Nugroho (2005), suggesting that the condition of epidermis surface and leaf structure affected feed preferences of larvae. The larvae of *A. atlas* used in this study had been well adapted to feed and tea leaves available in plantation for tens of generations.

Difference types and structure of leaves used as feed treatment during the study would probably affect feed consumption, as well as feed digestibility. Data presented in Table 3 showed that feed digestibility was significantly influenced (P<0.05) by the types of feed and feeding frequency. The highest feed digestibility was found at the first instar (I1) (27.6±0.012%) and second instar (I2) (28.1±0.027%). The digestibility of guava leaves with the daily feeding of 4 times to larvae of instar 3 (I3) and instar 4 (I4) was similar (31.7±0.007%, and 35.5±0.008% respectively). In average, the digestibility of feed at the first instar (I1) was 0.071±0.006 g/larvae and second instar (I2) was 0.133±0.012 g/larvae (Table 4). Those revealed that the undigested feed by larvae given walnut leaves 4 times a day was quite high. The feed intake, digestibility, and the amount of undigested feed of larvae fed guava leaves increased along with the changing of growth to instar III and IV. Whereas, the digestibility of walnut leaves was low as the times of feeding lesser than 4 times per day.

Data presented in Table 5 suggested that weight gain of *A. atlas* was affected by feeding treatments. The weight gain of larvae at the first instar of larvae given walnut and guava leaves for 4 times was significantly (P<0.05) higher than those of 3 times per day. Similar results were found at the second instar of larvae fed walnut leaves 4 times a day showed higher weight

Table 2. Feed consumption of *A. atlas* given fresh guava and walnuts leaves based on instar with different frequencies

Instar	Frequencies	n	Walnut leaves (g)	n	Guava leaves (g)
Instar I	3 times	75	0.304 ± 0.010^b	60	0.274 ± 0.012^c
	4 times	75	0.317 ± 0.015^a	65	0.284 ± 0.003^c
Instar II	3 times	67	0.425 ± 0.039^b	52	0.392 ± 0.012^c
	4 times	63	0.459 ± 0.015^a	60	0.392 ± 0.012^c
Instar III	3 times	40	0.815 ± 0.038^c	47	0.935 ± 0.040^b
	4 times	50	0.924 ± 0.050^b	55	1.000 ± 0.010^a
Instar IV	3 times	7	0.918 ± 0.125^b	10	1.132 ± 0.217^b
	4 times	10	1.025 ± 0.162^b	17	1.291 ± 0.132^a

Note: Mean in the same rows and columns with different superscript for the same instar differs significantly (P<0.05).

Table 3. Feed digestibility of *A. atlas* given guava and walnuts leaves based on instar with different frequency

Instar	Frequencies	n	Walnut leaves (%)	n	Guava leaves (%)
Instar I	3 times	75	25.90 ± 0.010^b	60	21.70 ± 0.003^c
	4 times	75	27.60 ± 0.012^a	65	22.60 ± 0.004^c
Instar II	3 times	67	26.10 ± 0.027^b	52	22.90 ± 0.009^c
	4 times	63	28.10 ± 0.027^a	60	23.40 ± 0.005^c
Instar III	3 times	40	27.50 ± 0.006^c	47	29.70 ± 0.007^b
	4 times	50	29.70 ± 0.005^b	55	31.70 ± 0.004^a
Instar IV	3 times	7	30.70 ± 0.006^b	10	32.50 ± 0.008^a
	4 times	10	31.50 ± 0.007^b	17	35.50 ± 0.008^a

Note: Mean in the same rows and columns with different superscript for the same instar differs significantly (P<0.05).

Table 4. Feed digested by *A. atlas* given guava and walnuts leaves based on instar with different frequency

Instar	Frequencies	n	Walnut leaves (g)	n	Guava leaves (g)
Instar I	3 times	75	0.067 ± 0.004^b	60	0.048 ± 0.003^c
	4 times	75	0.071 ± 0.006^a	65	0.050 ± 0.002^c
Instar II	3 times	67	0.111 ± 0.012^b	52	0.093 ± 0.014^c
	4 times	63	0.133 ± 0.012^a	60	0.095 ± 0.005^c
Instar III	3 times	40	0.229 ± 0.008^c	47	0.290 ± 0.037^b
	4 times	50	0.287 ± 0.023^b	55	0.331 ± 0.009^a
Instar IV	3 times	7	0.283 ± 0.042^c	10	0.378 ± 0.070^a
	4 times	10	0.323 ± 0.051^b	17	0.432 ± 0.044^a

Note: Mean in the same rows and columns with different superscript for the same instar differs significantly (P<0.05).

Table 5. Body weight gain of *A. atlas* given guava and walnuts leaves based on instar with different frequencies

Instar	Frequencies	n	Walnut leaves (g)	n	Guava leaves (g)
Instar I	3 times	75	0.033 ± 0.001^b	60	0.017 ± 0.001^d
	4 times	75	0.037 ± 0.001^a	65	0.019 ± 0.002^c
Instar II	3 times	67	0.102 ± 0.005^a	52	0.023 ± 0.001^b
	4 times	63	0.121 ± 0.004^a	60	0.033 ± 0.002^b
Instar III	3 times	40	0.209 ± 0.015^c	47	0.354 ± 0.060^b
	4 times	50	0.221 ± 0.019^c	55	0.527 ± 0.016^a
Instar IV	3 times	7	0.380 ± 0.035^b	10	0.428 ± 0.062^b
	4 times	10	0.499 ± 0.035^b	17	0.660 ± 0.215^a

Note: Mean in the same rows and columns with different superscript for the same instar differs significantly (P<0.05).

gains. Whereas, the average weight gain of larger larvae (I3 and I4) with feeding frequencies of 4 times was significantly higher than those of 3 times a day, and varied between types of feeds. Elzinga (2004) stated that more frequent of feedings may help to maintain the freshness and water content of feed leaves. In this regard, the growth of Lepidoptera larvae was highly depended on feed water content.

The size of *A. atlas* larvae based on their diameters (Table 5) was generally affected by types of feeds and feeding frequencies, especially at the first instar (I1) of which larvae fed both feed types for 4 times was significantly (P<0.05) higher than those of 3 feeding times (0.187 vs 0.033 cm). Difference results was found at the average diameter of larvae at the second (I2) instar which was similar at all treatments (Table 6). Along with growth development of larvae, the results become more varied; the larger the larvae size, the higher the feeding frequency resulted in higher size of body diameters, especially larva I3, given walnut leaves gave high result (0.806 cm). The adequate intake of good quality feed will improve growth and also the diameter of larvae size. According to Elzinga (2004), the growth of *A. atlas* larvae was much depended on some important aspects including feed availability and environmental conditions such as temperature, photoperiod and population density. Shortage of feed will certainly reduce the productivity of larvae. The overall results showed that 4 times feeding frequency per day could maintain the freshness of feed leaves given to larvaes, associated with short interval times between feedings. Intensification with good feeding management will increase feed consumption. Ahmad *et al*. (2006), stated that suitable feed for larvae should contain complete nutrition to produce good quality of silkworm, *A. atlas*.

Mortality rate of larvae as seen in Figure 2 was significantly high, especially at the late instars of I3 and I4. Difference leaves and feeding frequency significantly affected the survivals of larvaes, less frequency of feedings resulted in such high mortality. Despite the similar mortality rate found at the second instar (I2), the high mortality rate was also found at the I3 (approximately 40%). However, as the growth progressing, the extreme mortality rate was experienced

Table 6. Body diameter of *A. atlas* given guava and walnuts leaves based on instar with different frequencies

Instar	Frequencies	n	Walnut leaves (cm)	n	Guava leaves (cm)
Instar I	3 times	75	0.161 ± 0.017[b]	60	0.113 ± 0.017[c]
	4 times	75	0.187 ± 0.016[a]	65	0.117 ± 0.011[c]
Instar II	3 times	67	0.211 ± 0.024[a]	52	0.132 ± 0.008[b]
	4 times	63	0.267 ± 0.011[a]	60	0.137 ± 0.010[b]
Instar III	3 times	40	0.345 ± 0.030[c]	47	0.680 ± 0.063[b]
	4 times	50	0.365 ± 0.054[c]	55	0.806 ± 0.098[a]
Instar IV	3 times	7	0.287 ± 0.052[c]	10	0.440 ± 0.165[b]
	4 times	10	0.280 ± 0.052[c]	17	0.597 ± 0.094[a]

Note: Mean in the same rows and columns with different superscript for the same instar differs significantly (P<0.05).

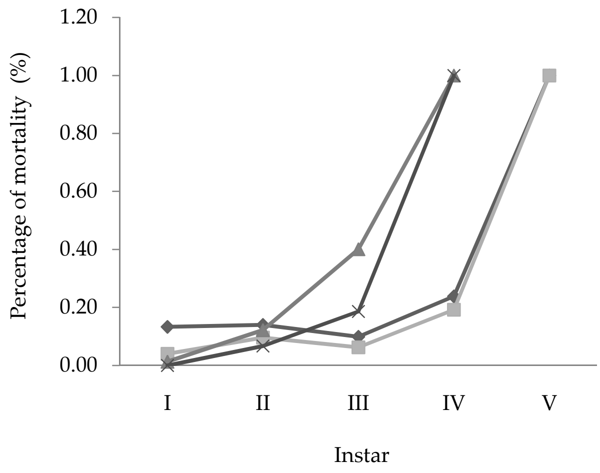

Figure 2. Mortality percentage of *A. atlas* given guava (-♦- : 3 times, -■- : 4 times) and walnuts (-▲- : 3 times, -x- : 4 times) leaves with different feeding frequencies.

with I4 and I5 larva's that achieved 100%, especially of those fed guava leaves with lesser feeding frequency. Less feed intake and hence feed digestibility of small larvae at early stage, might be responsible for the high percentage of mortality. Indrawan (2007) suggested that at early stage of growth development, small larvae required quality feeds with balanced nutrients and apropriate management to reduce mortality because young insect was more susceptible to death.

Regarding the environmental factors, temperature and humidity play important roles in the intensification process of *A. atlas*. During the study, the average temperature recorded in the morning was 27.44 ^{o}C, 28.21 ^{o}C in the afternoon and 27.52 ^{o}C in the evening. Relative humidity in the morning, afternoon and evening was 83.93%, 75.33% and 82.42% respectively and was considered high. Wulandari & Situmorang (2002), reported that temperature and humidity might cause stress to larvae, decreasing immunity, increasing susceptibility to diseases, and under extreme conditions may resulted in death. According to Nation (2008), the availability of sufficient waters, specifically in the fresh leaf feed, was extremely important. Keeping *A. atlas* in experimental rooms with such warm humidity and temperature, larvae will require adequate waters. Regarded as poikiloterm insect, fluctuations in temperature and humidity between times of days during rearing would have great effect on the survival rate of *A. atlas* larvae. Therefore, water becoming essential for larvae to adapt to the surrounding environment.

CONCLUSION

The optimum and efficient time sugested for mating is six hours resulting high egg production. The walnut leaves feed has better effect on the first and second instar of larvae, while the guava leaves affect the following instars. In terms of production, four times of feeding frequency per day has higher productivity and is recommended in the cultivation management of *A. atlas*.

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